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(54) Title: METHOD OF TREATMENT OF DISEASES BY DELETION OF T CELLS

(57) Abstract

This invention includes the discovery that chronic exposure to the superantigen (SAg) staphylococcal enterotoxin A (SEA) causes almost complete deletion of target T cells *in vivo*. Mice were either acutely or chronically exposed to varying doses of SEA, and the relative level of T cells bearing SEA-reactive V$\beta$ elements was followed over time in lymphocytes purified from peripheral blood, lymph nodes, mesenteric lymph nodes, and spleen. Acute exposure caused the disappearance of 50-70 % of reactive T cells. Chronic exposure caused almost complete deletion of target T cells. Deletion was evident even in animals treated with very low doses of SEA, doses too small to cause any apparent T cell proliferation. Thus, proliferation does not appear to be a prerequisite for peripheral deletion of T cells and peripheral tolerance may be achieved by chronic exposure to low levels of antigen without prior cell division.

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# METHOD OF TREATMENT OF DISEASE BY DELETION OF T CELLS

## FIELD OF THE INVENTION

This invention relates to the field of immunology. Specifically, this invention describes a method of treating diseases mediated by T cells of Vβ type by deletion of specific T cell populations. This invention is premised on the discovery that chronic exposure to a superantigen causes almost complete deletion of target T cells in vivo.

## BACKGROUND

The ability of an animal to respond to a particular immune challenge is dependent upon many factors, including the repertoire of mature T cells. T cells are a heterogeneous population of cells derived from the thymus which circulate through the blood and lymphatic vessels of the body. They function to detect and react against foreign invaders through a recognition system called the T cell antigen receptor (TCR). T cells respond to foreign antigens presented in the context of molecules of the major histocompatibility complex (MHC) or to non-self MHC molecules by proliferating and by the generation of effector cells with helper or cytolytic functions (Borst et al. (1987) J. Immunol. 139:1952).

Before helper T cells can recognize conventional protein antigens, the proteins must first undergo processing by macrophages or other antigen-presenting cells. These cells essentially swallow antigens and chop them into peptides. The presenters then display the peptide antigens at the cell surface in combination with MHC molecules. Once an antigen is displayed, the few helper T cells in the body that bear receptors for that particular peptide link up with it.

The TCR consist of two proteins chains, α and β, each chain containing a constant and a variable domain. The variable domains are encoded in two (α) or three

($\beta$) different gene segments (variable (V), diversity (D), and joining (J)) (Siu et al. (1984) Cell 37:393; Yanagi et al. (1985) Proc. Natl. Acad. Sci. USA 82:3430). In each T cell, the combination of V, D, and J domains of both the $\alpha$ and $\beta$ chains participates in antigen recognition in a manner which is uniquely characteristic of that T cell and defines a unique binding site. T cell TCR diversity is created through somatic rearrangement (Yanagi et al. (1984) Nature 308:145; Hedrick (1984) Nature 308:149; Siu et al. (1984) supra; Arden et al. (1985) Nature 316:783). The T cell repertoire is shaped by a complex series of positive and negative selection events (Blackman et al. (1989) Science 244:214).

Recently, it has been shown that a novel class of antigens, denoted superantigens (SAg), can have profound effects upon the T cell repertoire (Herman et al. (1991) Ann. Rev. Immunol. 9:745). Unlike conventional antigens, which must be processed by antigen presenting cells, SAgs are stimulatory in their native conformation. Indeed, the mitogenic effects of SAgs are destroyed by conventional antigen processing (Fraser (1989) Nature 339:221). Instead of interacting with the TCR by binding in the groove of an MHC molecule, like normally processed antigens, SAg bind to lateral faces of both the TCR and MHC molecules, forming a bridge between these cell-surface molecules on apposing cells. Selective activation of T cells by SAg is determined primarily by the V$\beta$ element of the TCR of the responding cells, independent of the specificity of that receptor for any particular MHC-processed antigen.

Superantigens from both viral and bacterial sources have been identified. Viral sources include the endogenous and exogenous mouse mammary tumor viruses (MMTV), which have been shown to have strong effects on the T cell repertoire in the mouse (Acha-

Orbea et al. (1991) Nature 350:207;, Dyson et al. (1991) Nature 349:531; Marrack et al. (1991) Nature 349:524; Woodland et al. (1991) Nature 349:529; Choi et al. (1991) Nature 350:203). Best characterized of the bacterial superantigens are the toxins from Staphylococcus aureus (Marrack and Kappler (1990) Science 248:705; Lee and Schlievert (1991) Curr. Top. Microbiol. and Immunol. 174:1). Seven serologically distinct enterotoxins, in addition to toxic-shock syndrome toxin and the exfoliating toxins, are able to interact with a significant proportion of T cells in a Vβ-specific manner. The bacterial SAg can stimulate mature T cells of both $CD4^+$ and $CD8^+$ lineages.

Superantigens may contribute to autoimmune diseases, in which components of the immune system attack normal tissue. The process of deletion of T cells responsive to self, potentially harmful self-reactive T cells, is called tolerance or negative selection (Kappler et al. (1987) Cell 49:273; Kappler et al. (1988) Nature 332:35; MacDonald et al. (1988) Nature 332:40; Finkel et al. (1989) Cell 58:1047). The immune system normally deletes self-reactive T cells, but occasionally a few appear to escape the surveillance mechanism. It has been suggested that SAg's ability to arouse 20 percent of a person's T cell repertoire could lead to the undesirable replication of the few circulating T cells that recognize self (Paliard et al. (1991) Science 253:325; Johnson et al. (1992) Scientific American 266:92). T cells bearing certain Vβ types have been implicated in various autoimmune conditions, including arthritis and multiple sclerosis. These findings imply that the destructive cells might be activated by a superantigen that binds to the identified Vβ types (Paliard et al. (1991) supra; Johnson et al. (1992) supra).

Autoimmune diseases include a large number of disorders, including such neural diseases as multiple

sclerosis and myasthenia gravis, diseases of the joints, such as rheumatoid arthritis, attacks on nucleic acids, as observed with systemic lupus erythematosus, and such other diseases associated with various organs, as psoriasis, juvenile onset diabetes, Sjögren's disease, and thyroid disease. These diseases can have a variety of symptoms, which can vary from minor and irritating to life-threatening. For example, rheumatoid arthritis (RA) is a chronic, recurrent inflammatory disease primarily involving joints, and affects 1-3% of North Americans, with a female to male ration of 3:1. Severe RA patients tend to exhibit extra-articular manisfestations including vasculitis, muscle atrophy, subcutaneous nodules, lymphadenopathy, splenomegaly, and leukopenia. It is estimated that about 15% of RA patients become completely incapacitated.

Many investigative efforts have focused on developing methods for the treatment of autoimmune diseases. For example, European Patent Publication 340 109, entitled Anti-T-cell receptor determinants as autoimmune disease treatment, and U.S. Patent No. 4,550,086, issued October 29, 1985 to Reinherz et al., entitled Monoclonal antibodies that recognize human T cells, describe a method of detecting a particular sequence of the variable region gene of T cell receptors associated with a particular disease and treating the disease with antibodies to that sequence. U.S. Patent No. 4,886,743, issued December 12, 1989 to Hood et al., entitled: Diagnostic reagents based on unique sequences within the variable region of the T cell receptor and uses thereof, describes a method of diagnosing diseases based on the presence of T cells with a unique sequence in the Vβ region associated with a specific disease. PCT Patent Application Publication WO 90/06758 describes a method for detecting specific Vβ regions associated with RA, specifically, Vβ3, Vβ9,

and Vβ10, and for the treatment of RA with monoclonal antibodies which recognize Vβ3, Vβ9, and Vβ10.

USSN 07/827,540, filed January 28, 1992, entitled: Method for modifying T cell response, hereby specifically incorporated by reference, describes a method of treating superantigen-mediated diseases. Pre-exposure of an animal to a mutated superantigen molecule, such as Staphylococcal enterotoxin B (SEB) conferred protection against subsequent exposure to the deleterious effects of the wild-type toxin. The mutated superantigen molecule modifies the T-cell response to the wild-type toxin without modifying the B-cell response. PCT/US93/00839, filed January 28, 1993, entitled: Protective effects of mutated superantigens, hereby specifically incorporated by reference, further describes the protective effects of mutated superantigens and their ability to selectively delete or inactivate specific T-cell populations.

Several groups have reported the effect of both the endogenous and exogenous administration of SAg upon the TCR repertoire in mice (Acha-Orbea et al. (1991) supra; White et al. (1989) Cell 56:27; Rellahan et al. (1990) J. Exp. Med. 172:1091; Kawabe and Ochi (1991) Nature 349:245; Webb et al. (1990) Cell 63:1249; Rammensee et al. (1989) Nature 339:541). However, studies of viral SAg do not permit experimental manipulation of the dosage of SAg and are further clouded by the fact that the precise cellular and tissue distributions of these SAg are not known.

Published reports of in vivo T cell deletion mediated by exogenous SAg have focused on Staphylococcal enterotoxin B (SEB). As with the MMTV SAg, deletion or anergy mediated by SEB is preceded by expansion of SEB-reactive subsets (Rellahan et al. (1990) supra; Kawabe and Ochi (1991) supra; MacDonald et al. (1991) Eur. J. Immunol. 21:1963; Gonzalo et al. (1992) Eur. J. Immunol. 22:1007; Herrmann et al. (1992)

Eur. J. Immunol. 22:1935). All of these studies employed relatively high doses (> 10 ug) of SEB, administered in a single dose.

**BRIEF SUMMARY OF THE INVENTION**

The present invention is premised on the discovery that specific T cell populations can be deleted without prior induction of T cell proliferation. This discovery may be applied in the treatment of diseases mediated by Vβ-bearing T cells.

The inventors herein show that chronic intraperitoneal (i.p.) administration of low doses of a superantigen (SAg), in one example Staphylococcal enterotoxin A (SEA), results in deletion of T cells bearing Vβ elements specifically reactive to that SAg.

This invention includes a method for treating diseases mediated by T cells bearing specific Vβ elements comprising causing the deletion of said T cell populations without prior induction of said T cell populations. The method of the present invention includes the administration of substances that inhibit specific Vβ elements, including antibodies to specific Vβ elements.

This discovery may also be used to achieve peripheral tolerance to low levels of antigen without prior cell division. The present invention includes a method for inducing tolerance to peripherally expressed self antigens comprising chronic intraperitoneal administration of a low dose of a superantigen which does not result in induction of T cell proliferation.

**BRIEF DESCRIPTION OF THE FIGURES**

FIGURE 1 depicts the level of Vβ3 or Vβ11 use in $CD4^+$ or $CD8^+$ T cells from the lymph nodes, mesenteric lymph nodes, blood, and spleen, for up to 20 days after a single dose of SEA. B10.BR/SgSnJ mice were given

0.78(°), 3.1(•), 12.5(▲), or 50(■) ug SEA i.v. in 0.2 ml basal salt solution (BSS) on day 0. Control (BSS alone) and SEA-treated mice were sacrificed on days 0, 2, 4, 6, 9, 12, and 20. Each point represents data from a single animal, except for day 0 values, which are the averaged values from 6 BSS-treated control mice. FIGURE 1A-1D show the level of Vβ3 use in $CD4^+$ T cells. FIGURE 1E-1H show Vβ3 use among $CD8^+$ T cells. FIGURE 1I-1L show Vβ11 use among $CD8^+$ T cells.

FIGURE 2 depicts the expression of Vβ3 following acute exposure to low levels of SEA. B10.BR/SgSnJ mice received 0.008(°), 0.04(•), 0.2(▲), or 1.0(■) ug of SEA i.v. in 0.2 ml BSS. On days 2 and 10, Vβ use was analyzed among T cells. FIGURE 2A shows Vβ3 use in $CD4^+$ lymph node T cells. FIGURE 2B shows Vβ3 use in $CD8^+$ lymph node T cells.

FIGURE 3 depicts the depletion of Vβ3-bearing T cells as a result of chronic exposure to SEA. B10.BR/SgSnJ mice were given intraperitoneal injections of 0.008. 0.04, 0.2, or 1.0 ug SEA on day 0 and every other day thereafter. Control and SEA-treated mice were sacrificed on days 0, 2, 4, 6, 9, 12, and 16. The percentages of T cells bearing Vβ3, Vβ11, and Vβ14 were determined for T cells from the spleen, mesenteric lymph nodes, peripheral lymph nodes, and blood. Each point represents staining results from a single mouse, except for day 0 values, which are the averaged values from 6 BSS-treated control mice. FIGURE 3A-3D show Vβ3 use among $CD4^+$ T cells. FIGURE 3E-3H show Vβ14 use among $CD4^+$ T cells. FIGURE 3I-3L show Vβ3 use among $CD8^+$ T cells. FIGURE 3M-3P show Vβ11 use among $CD8^+$ T cells. FIGURE 3Q-3T show Vβ14 use among $CD8^+$ T cells.

**DETAILED DESCRIPTION OF THE INVENTION**

It is to be understood that both the foregoing

general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

The present invention includes a method for treating diseases mediated by T cells bearing specific Vβ elements by causing the deletion of those T cell populations. In one embodiment of the present invention, diseases mediated by specific Vβ-bearing T cells are treated by the deletion of those T cell populations -- without prior induction of T cell proliferation -- by chronic intraperitoneal administration of low doses of superantigen. In another embodiment of the present invention, diseases mediated by specific Vβ-bearing T cells are treated by the administration of substances inhibitory to specific Vβ elements, such as by the administration of Vβ antibodies.

The inventors of this invention have examined mice that were either acutely or chronically exposed to varying doses of SEA, and the relative level of T cells bearing SEA-reactive Vβ elements was followed over time in lymphocytes purified from peripheral blood, lymph nodes, mesenteric lymph nodes, and the spleen. Acute exposure caused the disappearance of 50-70% of reactive T cells. Chronic exposure caused almost complete deletion of target T cells. Deletion was evident even in animals treated with very low doses of SEA, doses too small to cause any apparent T cell proliferation. Thus, proliferation does not appear to be a prerequisite for peripheral deletion of T cells and peripheral tolerance may be achieved by chronic exposure to low levels of antigen without prior cell division. The present discovery may also be used as a method for inducing tolerance to peripherally expressed self antigens.

One embodiment of the present invention includes an extensive characterization of T cell proliferation

and deletion induced by the exogenous SAg SEA. Different doses of SEA were administered in either a single (acute) injection or repeated (chronic) doses. Proportionate use of several different TCR elements was followed over time in several different lymphoid compartments, for both CD4- and CD8-bearing T cells. A surprising finding of the present invention is that repeated administration of SEA, even in very small amounts, effectively deleted T cells bearing reactive Vβ elements ("target" Vβ elements) in the apparent absence of prior proliferation. Thus, peripheral tolerance may be achieved by chronic exposure to low levels of antigen without prior cell division, a situation which may mimic induction of tolerance to peripherally expressed self antigens.

The results of experiments described here dealing with acute exposure of animals to superantigens agree with those previously described (Kawabe and Ochi (1991) supra; Webb et al. (1990) supra; Jones et al. (1990) supra; Dannecker et al. (1991) supra; MacDonald et al. (1991) supra). Like the previous investigations, these studies found that acute exposure causes a rapid increase in T cells bearing target Vβs followed by a rapid decline to percentages which are in most cases about 30-50% of normal levels. Both the increases and declines occurred concomitantly in all lymphoid organs examined, therefore neither set of effects could have been due to migration of cells from one organ to another. The decline of a given T cell Vβ population seen in the present invention was found even in animals given very small doses of SEA, doses which were too small to cause any apparent prior proliferation.

It has been shown that a large number of diseases and medical conditions have a specific Vβ footprint. The "footprint" usually consists of elevated levels of certain Vβ elements. Although the role of the elevated Vβ populations in the etiology of these diseases is not

known, it must be assumed that the Vβ specific population plays some role in the mediation of the disease or medical condition.

The present invention provides a method for reducing levels of T-cell populations having a specific Vβ element. There exists a body of information regarding known superantigens as well as the specific Vβ elements affected by the superantigen. The present invention may be used to advantage in several ways. For example, the chronic administration of a superantigen will delete the same population of Vβ elements that are expanded by the exposure to relatively large quantities of the superantigen. The chronic low level administration can serve, therefore, as a type of vaccination to the superantigen itself.

In another embodiment of the invention, the superantigen giving rise to or mediating the given disease or medical condition is not known. However, since the Vβ "footprint" of the disease is known, it is possible to select one of the known superantigens that has common Vβ elements in its footprint. By this means, any number of diseases may be prevented or treated by the T-cell deletion process of this invention.

The present invention anticipates the administration to a patient of a SAg to reduce certain T cell populations containing a given Vβ element. In the normal case, the Vβ element that will be effected will correspond to the Vβ element (or elements) that is activated upon exposure to a large dose of the SAg. However, it is also contemplated that in some instances the chronic administration of a natural SAg may lead to the deletion of T cell populations containing Vβ elements that are not effected by larger doses of the SAg.

In addition, the present invention includes the administration of modified and mutated superantigens.

The preparation and characterization of such modified and mutated superantigens are described in detail in U.S. patent application serial number 07/827,540 filed January 28, 1992 entitled "Method for Modifying T Cell Response", and PCT application PCT/US93/00839, filed January 28, 1993, entitled "Protective Effects of Mutated Superantigens". Both of these applications are specifically incorporated herein by reference. Again, it is anticipated that certain modified and mutated superantigens will have the ability to delete certain T cell populations based on common Vβ elements. In some instances the T cell populations deleted upon chronic administration of the mutated superantigen will be the same as would be deleted upon chronic administration of the non mutated superantigen. However, in other cases the mutations will alter the specificity of the superantigen and T cell populations with different Vβ elements will be deleted. It would be well within the skill and knowledge of one skilled in the art to analyze the mutated superantigens to determine which T cell populations will be deleted by the chronic administration of such mutated superantigens.

According to this invention, diseases having a specific Vβ footprint -- generally superantigen initiated or mediated diseases -- may be prevented or treated by the administration of a superantigen in a manner that leads to the deletion of certain Vβ populations without any initial T-cell proliferation. In the preferred embodiment, the superantigen is administered chronically in low dosages. "Chronically" is defined as more than one administration, and more specifically, is generally meant to include periodic administrations (e.g., hourly, twice daily, daily, weekly, etc.) for a period of time sufficient to lead to T-cell Vβ specific deletion.

By "low dosages" is meant a dosage of superantigen of less than 100 ug/kg, and preferably less than 50

ug/kg. Since the amount of superantigen necessary to lead to deletion without causing initial T-cell proliferation may very among the superantigens, the low level of dosage may be defined as anything less than the amount of superantigen administration that gives rise to T-cell proliferation.

The method of the present invention includes therapeutic compositions of superantigen which may be administered parenterally by injection, although other effective administration forms, such as intraarticular injection, inhalant mists, orally active formulations, transdermal iontophoresis or suppositories, are also envisioned. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment it is envisioned that the carrier and the superantigen constitute a physiologically-compatible, slow-release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the superantigen. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or

requiring reconstitution immediately prior to administration. The preferred storage of such formulations is at temperatures at least as low as 4°C and preferably at -70°C. It is also preferred that such formulations containing the superantigen are stored and administered at or near physiological pH. It is presently believed that administration in a formulation at a high pH (i.e. greater than 8) or at a low pH (i.e. less than 5) is undesirable.

The manner of administering the formulations containing superantigen for systemic delivery is via subcutaneous, intramuscular, intravenous, intranasal or vaginal or rectal suppository. Preferably, administration is by intraperitoneal injection.

In contrast to acute exposure, chronic exposure to SEA resulted ultimately in the almost complete disappearance of all $CD4^+$ and $CD8^+$ T cells bearing V$\beta$3 in all tissues analyzed. This result was seen at all doses of SEA tested. As discussed above, since the disappearance occurred in all organs tested, it did not reflect a redistribution of $V\beta3^+$ cells. The disappearance occurred far too quickly to be explained by thymic depletion (i.e., the death of SEA-reactive cells in the thymus, with the subsequent dilution of the peripheral pool of mature T cells). It is also unlikely that the deletion seen here is actually the result of a downmodulation of TCR levels in SEA reactive cells, as overnight culture of cells removed from mice treated with SEA did not lead to a reappearance of $V\beta3^+$ cells. Therefore, chronic exposure to superantigens must cause the death of target peripheral T-cells. The demonstration that low, chronic levels of exogenous SAg are particularly efficient at causing the deletion of SAg-reactive T-cells suggests that such treatment is therapeutically useful in the treatment of diseases mediated by T-cells of known V$\beta$ type. Further, such treatment may be for

the induction of post-thymic T-cell tolerance.

Although not limited by theory, it has been suggested that superantigens cause the death of peripheral T cells because they deliver an overpowering stimulus to such cells, a stimulus which drives proliferation and then death (Kawabe and Ochi (1991) supra; Webb et al. (1990) supra). This explanation may account for the deletion of Vβ3-bearing cells observed after the administration of high doses of SEA, but it cannot account for the profound deletion of $V\beta3^+$ cells caused by very low amounts of SEA given chronically. Mice receiving 0.008 ug SEA every other day contained very few $CD4^+/V\beta3^+$ T cells 7 days after the start of treatment with little sign of initial T cell division. This treatment certainly did not cause large scale proliferation of target T cells, and the results therefore suggest that a massive proliferation response is not an essential prerequisite to SAg-mediated T cell deletion.

The results described below do not explain the mechanism of how prolonged exposure to low doses of SAg causes T cell death. Again, not limited by theory, most of the Class II proteins in a normal animal are expressed on small resting B cells, so perhaps the T cells die because they encounter SEA bound to the surface of nonprofessional antigen presenting cells, cells which lack BB1 and other accessory molecules thought to be necessary for the normal activation of T cells (Jenkins et al. (1987) Immunol. Rev. 95:113; Jenkins et al. (1991) J. Immunol. 147:2461).

Two Vβ elements (Vβ3 and Vβ11) have previously been characterized as SEA-reactive (Callahan et al. (1990) J. Immunol. 144:2473). Treatment with SEA had slightly different effect on T cells bearing Vβ11 rather than Vβ3. $CD8^+/V\beta11$-bearing cells did not increase in numbers as much as $CD8^+/V\beta3$-bearing cells during chronic treatment with the lowest doses of SEA,

nor were the Vβ11$^+$ cells as effectively deleted. This difference may simply reflect a lower affinity of SEA for Vβ11 than for Vβ3, as the toxins have been shown to have varying affinities for their different Vβ targets (Fleischer and Schrezenmeier (1988) J. Exp. Med. 167:1697; Kappler et al. (1992) J. Exp. Med. 175:387). Such an explanation would predict that the dose response curve of Vβ11$^+$ T cells to SEA should be shifted with respect to that of Vβ3-bearing cells; however, this was not the observed result. Chronic SEA treatment did not cause overwhelming deletion of CD8$^+$/Vβ11$^+$ T cells at any dose, even though higher doses caused excellent initial expansion of such cells. The difference in response of Vβ11-bearing cells may be due to the fact that B10.BR mice contain at least one MTV which expresses an endogenous Vβ11-reactive SAg (Woodland et al. (1991) supra; Bill et al. (1989) J. Exp. Med. 169:1405). It may also be that the CD8$^+$/Vβ11$^+$ cells in the B10.BR mouse are refractory to SEA-mediated deletion because they are in an unusual state of activation or anergy due to the expression of MTV-9 (and perhaps MTV-8) encoded SAgs.

Although, as shown in previous references, the effects of these Class II-associated bacterial SAgs on the CD4$^+$ and CD8$^+$ T cell populations were similar, there were some subtle differences in the responses to SEA of these two types of cells. For example, the CD4$^+$ cells did not disappear as rapidly as the CD8$^+$ cells during chronic treatment with SEA. It is possible that these differences are related to the ability or inability of the accessory molecules CD4 or CD8 to join the TCR/SAg/Class II complex, where these accessory molecules could serve to enhance the stability of the interactions within the complex, and/or to provide secondary intracellular signals to the responding T cell. It is also possible that the two subsets of cells simply have inherently different thresholds of

activation - in effect, they could be "programmed" to respond differently to marginal or suboptimal signals.

Finally, there were also subtle differences in the responses of cells bearing the same Vβ in different organs. For example, T cells bearing Vβ3 expanded slightly in spleen but not lymph nodes in response to chronic i.p. treatment with low amounts of SEA. This observation could be due to migration of these cells to the spleen from other locations or to uneven distribution of SEA in these animals. Alternatively, the activation state of T cell populations in different lymphoid compartments may differ, leading to differences in their responses to marginal levels of SAg. A related possibility is that the antigen-presenting cells found in different locations deliver different ancillary signals, resulting in differences in the response of SAg-reactive T cells. The present data do not distinguish between the possibilities.

The following examples serve to explain and illustrate the present invention, and are not to be construed as limiting of the invention in any way. Example 1 describes the materials and method of the present invention. Example 2 describes the result of acute exposure of mice to the superantigen toxin SEA on Vβ use. Example 3 describes the effects of chronic superantigen administration on target Vβ expression, and shows that extremely low doses of SEA given chronically preferentially deleted a specific population of Vβ T cells.

**Example 1. MATERIALS AND METHODS**

Animals. B10.BR/SgSnJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in the animal care facilities of the National Jewish Center for Immunology and Respiratory Medicine. Mice were between 6 and 12 weeks of age. For some experiments, animals were given a single dose of SEA,

administered intravenously (i.v.) in 0.2 ml balanced salts solution (BSS); the injection date was day 0. In other experiments, SEA was administered intraperitoneally (i.p.) every other day in 0.2 ml BSS, starting on day 0.

Antibodies. The following monoclonal antibodies (mAbs) to mouse antigens were used in analysis of the TCR repertoire: GK1.5, rat anti-CD4 (Dialynas et al. (1983) J. Immunol. 133:2445); rat anti-CD8 (Ledbetter and Herzenberg (1979) Immunol. Rev. 47:63; KJ25-606.7, hamster anti-V$\beta$3 (Pullen et al. (1988) Nature 335:796), 14-2, rat anti-V$\beta$14 (Liao et al. (1989) J. Exp. Med. 170:135), RR3-15, rat anti-V$\beta$11 (Bill et al. (1989) J. Exp. Med. 169:1405), H57-597, hamster anti-$\alpha\beta$ (Kubo et al. (1988) J. Immunol. 142:2736). All anti-TCR reagents were biotinylated, and phycoerythrin-conjugated streptavidin (Tago Inc., Burlingame, CA) was used as a secondary reagent. Anti-CD4 and anti-CD8 mAbs were directly fluoresceinated using fluorescein isothiocyanate (Sigma, St. Louis, MO).

Staining and FACS Analysis. SEA-treated mice were tail-bled, the blood collected into BSS containing 1% heparin, then mice were sacrificed by cervical dislocation. Peripheral lymph nodes (inguinal, brachial and axillary), mesenteric lymph nodes, and spleen were harvested separately. Lymphocytes were released from organs by passage through nylon mesh. Peripheral blood and spleen samples were treated with buffered ammonium chloride to lyse erythrocytes, followed by two washes with BSS. All samples were passed over nylon wool (Julius et al. (1973) Eur. J. Immunol. 3:645) prior to staining, to enrich for T cells, and were resuspended at concentrations from $10^7$-$10^8$ cells/ml, depending upon yield. Peripheral, mature T cells were stained as described by Callahan et al. (1990) supra). Two-color FACS analysis was performed on a Coulter EPICS C (Coulter Electronics, Hialeah, FL)

with a 488 nm argon ion laser.

The proportion of T cells bearing a given Vβ element was determined relative to net $\alpha\beta^+$ cells. In analyzing CD4 and CD8 usage, it was assumed that cells which stained positive for TCR but not CD8 were $CD4^+$, and that conversely, $TCR^+$ cells negative for CD4 were $CD8^+$. In all cases, the sum of ($CD4^+$) and ($CD8^+$) cells in a sample matched the net $\alpha\beta$ $TCR^+$ cells to within 1-2%. Proportional use of specific Vβ elements within the $CD4^+$ population was calculated from the ratio of (net specific Vβ-bearing $CD4^+$ cells)(net $\alpha\beta$ TCR-bearing $CD4^+$ cells); similar calculations were used for the $CD8^+$ population. All calculations included a corrector for background levels of fluorescence, obtained by staining cells with the secondary reagent, PE-avidin, alone. In analyzing peripheral lymph node, spleen, and mesenteric lymph node cells, a minimum of 20,000 cells were analyzed for each staining. Between 5,000 and 20,000 cells per staining were analyzed for peripheral blood samples.

Reagents. Staphylococcal enterotoxin A (SEA) was purchased from Toxin Technologies (Madison, WI). Lyophilized material was resuspended in balanced salt solution (BSS) and sterilized by filtration.

Example 2. Effect of Acute Exposure of SEA on Vβ Use.

Several studies have described the effect on mice of exposure to viral SAg (Webb et al. (1990) supra; Dannecker et al. (1991) J. Immunol. 146:2083; Gonzalo et al. (1992) supra) and bacterial SAg (Rellahan et al. (1990) supra; Kawabe and Ochi (1991) supra; MacDonald et al. (1991) Eur. J. Immunol. 21:1963; Herrmann et al. (1992) supra). However, with the exception of a single report of SEA-induced allergy (MacDonald et al. (1991) supra), little information has been available regarding the results of in vivo exposure to SEA.

In order to do a more systematic analysis of the in vivo effects of SEA on the T cell repertoire, animals were given single i.v. injections of SEA at varying doses. At various times following this injection, animals were sacrificed, and Vβ use among the T cells of various lymphoid compartments was determined. The results of this timecourse are displayed in Figure 1, while levels of Vβ expression after 12 days of treatment are also listed in Table I.

T cells bearing Vβ3, one of the target Vβs for SEA (Callahan et al. (1990) supra), increased dramatically in percentage in all lymphoid compartments examined. A two- to three-fold increase in the percentage of Vβ3-bearing cells was found in both $CD^+$ (Figure 1A) and $CD8^+$ (Figure 1B) populations of blood, peripheral and mesenteric lymph node T cells. This enrichment was even more pronounced among splenic T cells, were the proportion of cells bearing Vβ3 reached levels as high as six-fold above control levels within two days. Between 2 and 4 days after injection of SEA, the percentage of Vβ3-bearing cells in all organs dropped quickly, and decreased to below control levels in most cases between days 6 and 9. In no case did the Vβ3-bearing cells disappear completely.

The response of cells bearing Vβ11, another Vβ engaged by SEA, was similar (Figure 1C). There are very few Vβ11+ cells in the $CD4^+$ compartment of B10.BR mice, therefore only $CD8^+$ cells were analyzed. The response of these cells was similar to that of the $CD8^+/V\beta3^+$ population, although some subtle differences are apparent. For instance, while the expansion of $CD8^+/V\beta3^+$ cells was comparable in all compartments examined, the expansion of $CD8^+/V\beta11^+$ cells was more pronounced in the peripheral blood (note the scale of the Y axis) Furthermore, while the highest dose of SEA employed here led to maximal expansion of the $CD8^+/V\beta3^+$ subset, the expansion of the $CD8^+/V\beta11^+$ population

appears to be attenuated at higher doses of SEA.

Changes in the percentages of control T cells bearing Vβ 14 (not shown) reflected the expansion and deletion of the SEA reactive populations, i.e., in mice which had undergone considerable expansion or deletion of Vβ3- and Vβ11-bearing T cells, Vβ14-bearing T cells (and presumably those bearing other non-SEA-reactive Vβs) were reduced or increased in percentage compensatorily.

In a second series of experiments mice were exposed to even lower doses of SEA, given acutely (Figure 2). Animals were assayed for proliferation of target T cells 2 days later and for deletion of target T cells 10 days later. Increased numbers of $V\beta3^+$ cells bearing either CD4 or CD8 were seen by day 2 at all doses of SEA given except the lowest, 0.0008 ug, where no change was seen in the $CD4^+/V\beta3^+$ population. Significant deletion of $CD4^+/V\beta3^+$ T cells had occurred by day 10 at all doses used; indeed, the lower doses seemed to lead to better deletion.

Example 3. Effect of Chronic Exposure of SEA on Vβ Use.

In an attempt to mimic the effects of chronic exposure to peripheral self antigens, mice were challenged with continuous, low-level doses of SAg. Adult B10.BR mice were injected i.p. with 0.008, 0.04, 0.2, or 1.0 ug of SEA every other day. At intervals animals were sacrificed, and the TCR repertoire in lymph nodes, spleen and peripheral blood analyzed to determine the in vivo effects of chronic SEA exposure.

Chronic exposure to higher doses of SEA had the same effects as those resulting from acute exposure to similar amounts of SEA. Target T cells first proliferated and then disappeared. The deletion caused by chronic exposure was far more profound, however, with hardly any detectable Vβ3-bearing cells left in either the $CD4^+$ or $CD8^+$ subsets within 9 days of the start of treatment (Figures 3A and 3C). Levels of VB expression after 12 days of treatment are listed in Table II.

Extremely low amounts of SEA given chronically also deleted $V\beta3^+$ T cells as effectively as higher doses. In contrast to the deletion seen following either acute treatment or treatment with higher chronic doses, this deletion occurred in the apparent absence of preceding proliferation, particularly among the $CD4^+$ subset. Moreover, deletion began relatively early in the response. Thus, Vβ3-bearing T cells began to drop in percentage as early as day 2 in the mesenteric lymph nodes and peripheral blood of mice given 0.04 or 0.008 ug SEA every other day although Vβ3-bearing $CD4^+$ cells still did increase in percentage somewhat in spleen prior to their deletion. Whether this local increase represents proliferation of splenic $CD4^+/V\beta3^+$ cells, or a migration of these cells from other tissues to the spleen cannot be determined from these data. These results differ from those presented in Figure 2, where

similar low doses of SEA did not cause deletion of $V\beta3^+$ cells after two days. Presumably, this difference is due to the different routes of administration used in the two experiments, i.v. in the experiments shown in Figure 2, and i.p. in the experiments shown in Figure 3. These two protocols presumably result in different patterns of SEA distribution within the recipient animals.

The effects of chronic SEA treatment on $V\beta11$-bearing T cells were somewhat different. Although higher doses cause these cells to divide, lower chronic doses of SEA (0.008-0.04 ug) did not result in expansion of the $CD8^+/V\beta11^+$ subset, even though expansion of the $CD8^+/V\beta3^+$ subset was apparent at theses doses. Furthermore, although some deletion of these cells was seen, especially at higher doses, chronic SEA treatment did not cause profound systemic deletions as are seen in the $V\beta3^+$ population.

The depletion of T cells bearing target $V\beta$s caused by chronic exposure to SEA is not permanent. In B10.BR/SgSnJ mice treated with nine injections of SEA on alternate days over a 16 period, and then rested without further SEA treatment for 1 month, the percentages of $V\beta3$- and $V\beta11$-bearing T cells in the spleen rose (Table III); similar results were seen among lymph node T cells (data not shown). The observation that T cell recovery was slower in animals treated chronically with higher amounts of SEA, despite the fact that the deletion of target cells was comparable at all doses at day 16, suggests that SEA may accumulate and persist <u>in vivo</u> for periods of several weeks.

Analysis of $V\beta$ usage was performed as described in Example 1. Data are presented as percentages of values obtained from control mice (no SEA treatment) analyzed on the same day. Data shown are for splenic T cells.

The observed recovery is probably due to replacement by cells newly generated in the thymus in the absence of SEA, since it has been observed that recovery of this type does not occur in thymectomized animals released form exposure to superantigen (data not shown). The observation that T cell recovery was slower in animals treated chronically with higher amounts of SEA, despite the fact that the deletion of target cells was comparable at all doses at day 16, suggests that SEA may accumulate and persist in vivo for periods of several weeks.

TABLE I. EXPRESSION OF TARGET Vβs AMONG SPENIC T CELLS AFTER 12 DAYS OF IN VIVO ACUTE EXPOSURE TO SEA.

| Population | 50.0 ug | 12.5 ug | 3.1 ug | 0.78 ug | Control |
|---|---|---|---|---|---|
| $CD4^+/V\beta3^+$ | 5.11% | 3.98% | 3.97% | 2.72% | 5.00% (0.29) |
| $CD8^+/V\beta3^+$ | 3.10% | 2.81% | 2.73% | 2.47% | 2.34% (0.22) |
| $CD8^+/V\beta11^+$ | 1.40% | 1.55% | 1.09% | 1.58% | 2.25% (0.09) |
| $CD4^+/V\beta14^+$ | 8.17% | 7.18% | 7.24% | 8.02% | 7.40% (0.33) |
| $CD8^+/V\beta14^+$ | 5.46% | 5.91% | 6.21% | 7.67% | 6.53% (0.30) |

TABLE II. EXPRESSION OF TARGET Vβs AMONG SPENIC T CELLS AFTER 12 DAYS OF IN VIVO CHRONIC EXPOSURE TO SEA.

| Population | 1.0 ug | 0.2 ug | 0.04 ug | 0.008 ug |
|---|---|---|---|---|
| $CD4^+/V\beta3^+$ | 2.34% | 1.29% | 0.92% | 0.88% |
| $CD8^+/V\beta3^+$ | < 0.1% | < 0.1% | < 0.1% | 0.15% |
| $CD8^+/V\beta11^+$ | 0.89% | 1.80% | 2.14% | 1.95% |
| $CD4^+/V\beta14^+$ | 9.37% | 8.79% | 7.76% | 8.58% |
| $CD8^+/V\beta14^+$ | 6.91% | 6.24% | 5.65% | 6.44% |

TABLE III. RECOVERY OF SPLENIC T CELLS BEARING TARGET Vβs AFTER RELEASE FROM CHRONIC EXPOSURE TO SEA.

| Population | 1.0 ug | 0.2 ug | 0.04 ug | 0.008 ug |
|---|---|---|---|---|
| $CD4^+/V\beta3^+$ | 35.5% | 45.0% | 42.5% | 59.8% |
| $CD8^+/V\beta3^+$ | 10.7% | 37.3% | 24.3% | 58.1% |
| $CD8^+/V\beta11^+$ | 34.2% | 54.2% | 96.8% | 95.8% |
| $CD4^+/V\beta14^+$ | 95/5% | 100.1% | 96.1% | 97.6% |
| $CD8^+/V\beta14^+$ | 109.8% | 108.8% | 94.9% | 106.6% |

**CLAIMS**

1. A method for treating diseases mediated by T cells bearing specific Vβ elements comprising causing the deletion of said T cell populations without prior induction of said T cell populations.

2. The method of claim 1 comprising chronic intraperitoneal administration of a low dose of a superantigen.

3. The method of claim 1 comprising chronic intraperitoneal administration of a low dose of a mutated superantigen.

4. The method of claim 1 comprising administration of an inhibitor of said Vβ elements.

5. The inhibitor of claim 4 wherein said inhibitor is comprised of an antibody to said Vβ element.

6. A method for inducing tolerance to peripherally expressed self antigens comprising chronic intraperitoneal administration of a low dose of a superantigen which does not result in induction of T cell proliferation.

7. The method of claim 6 wherein said superantigen is a mutated superantigen.

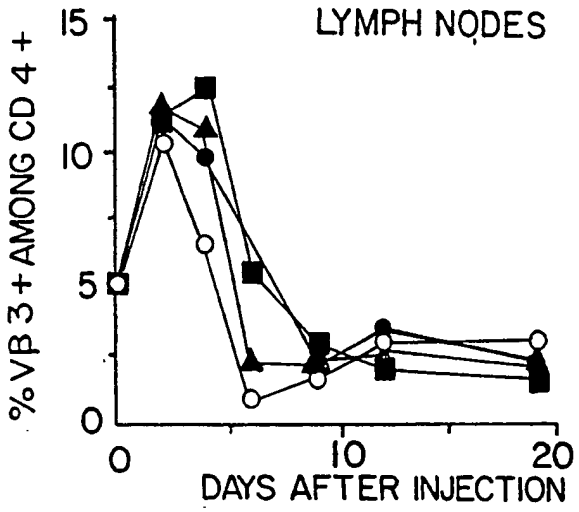


FIG. 1A

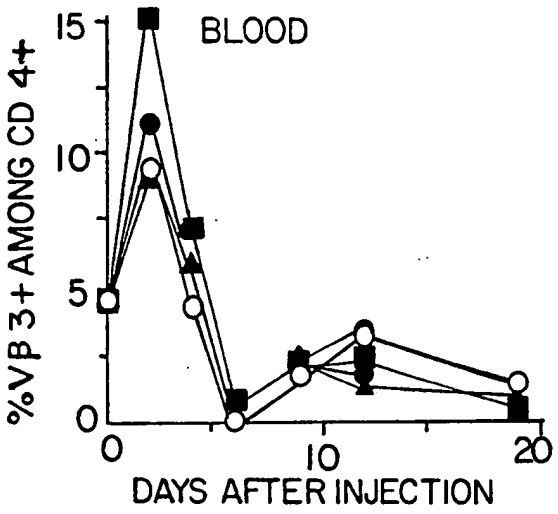


FIG. 1B

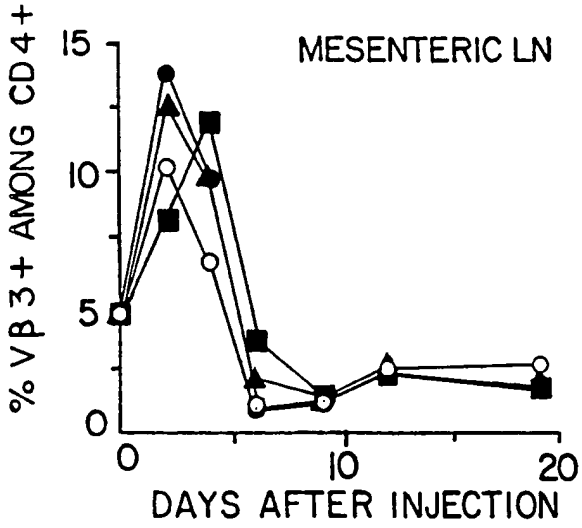


FIG. 1C

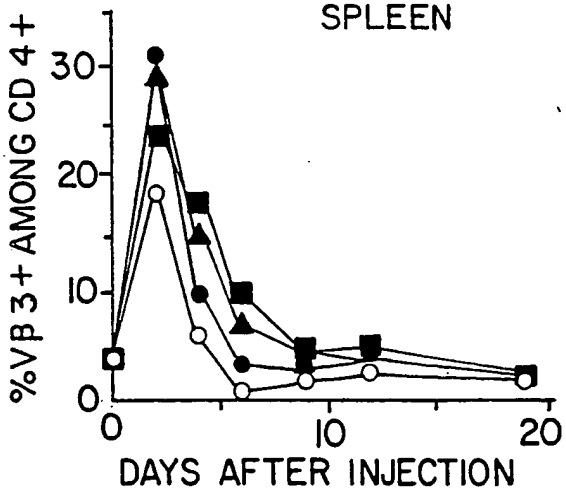


FIG. 1D

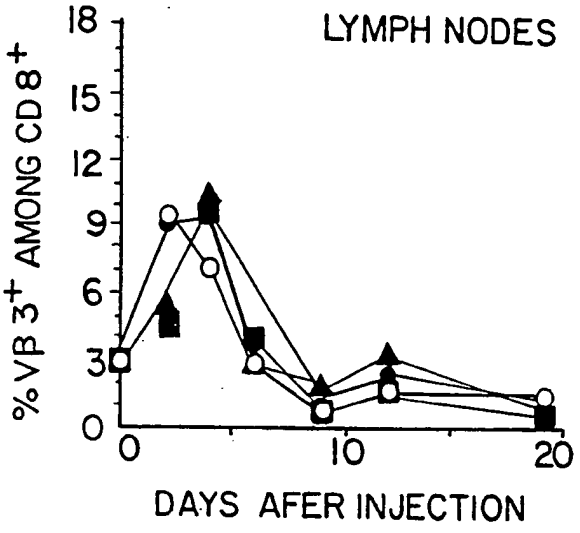


FIG. 1E

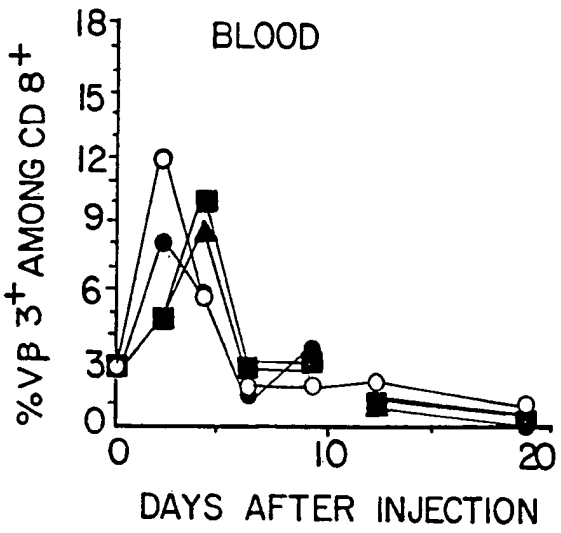


FIG. 1F

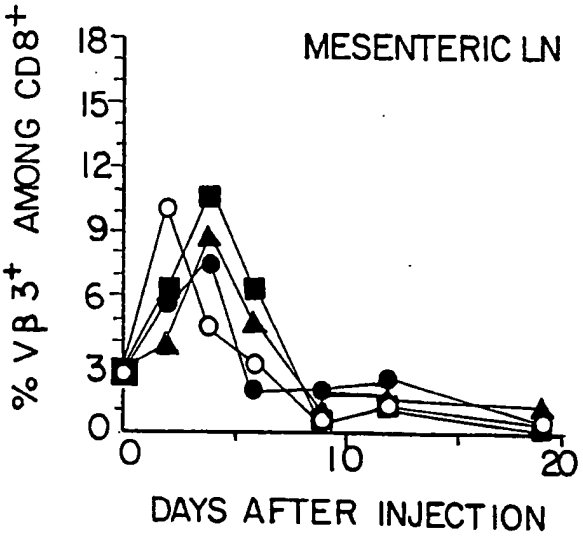


FIG. 1G

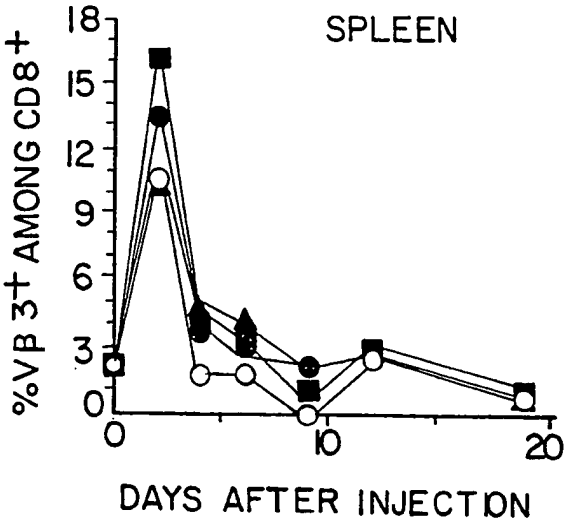


FIG. 1H

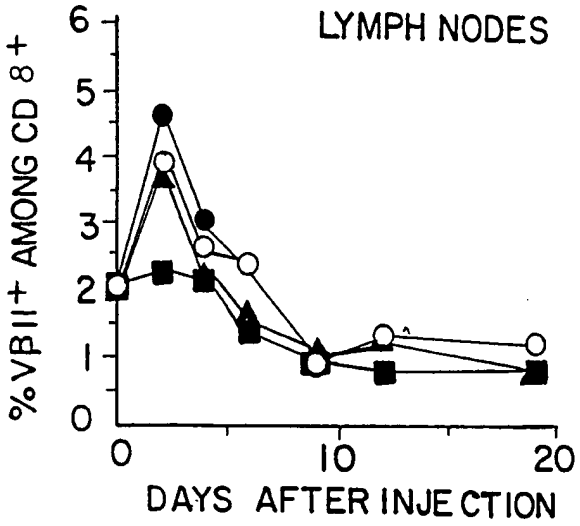


FIG. II

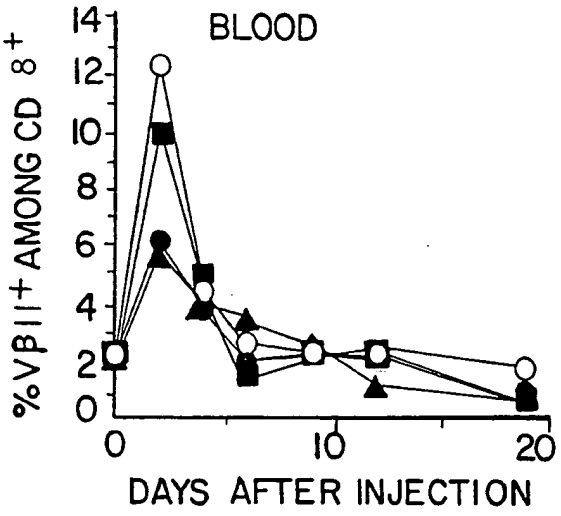


FIG. IJ

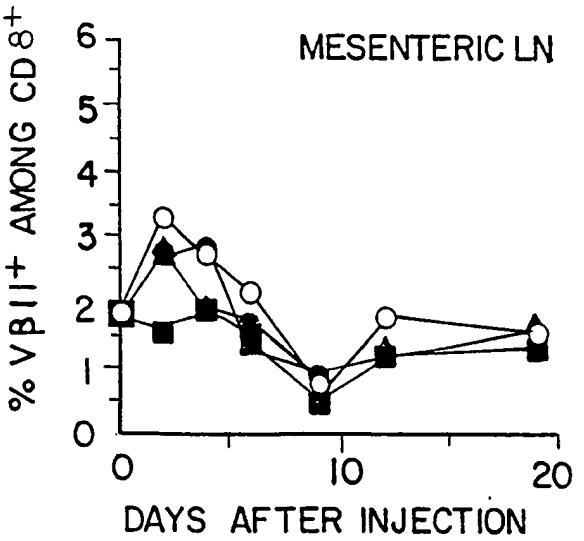


FIG. IK

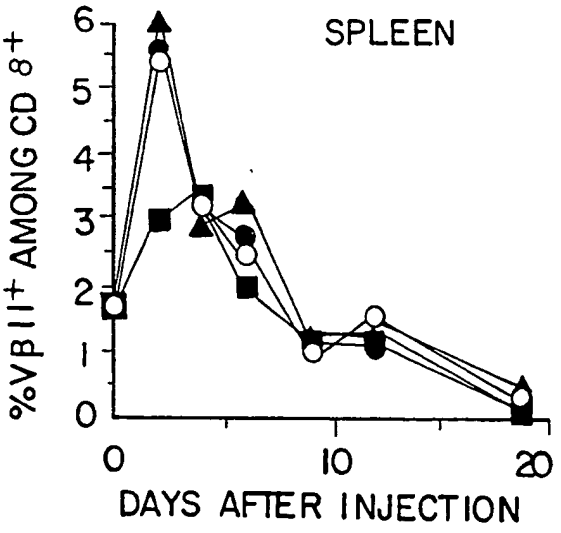


FIG. IL

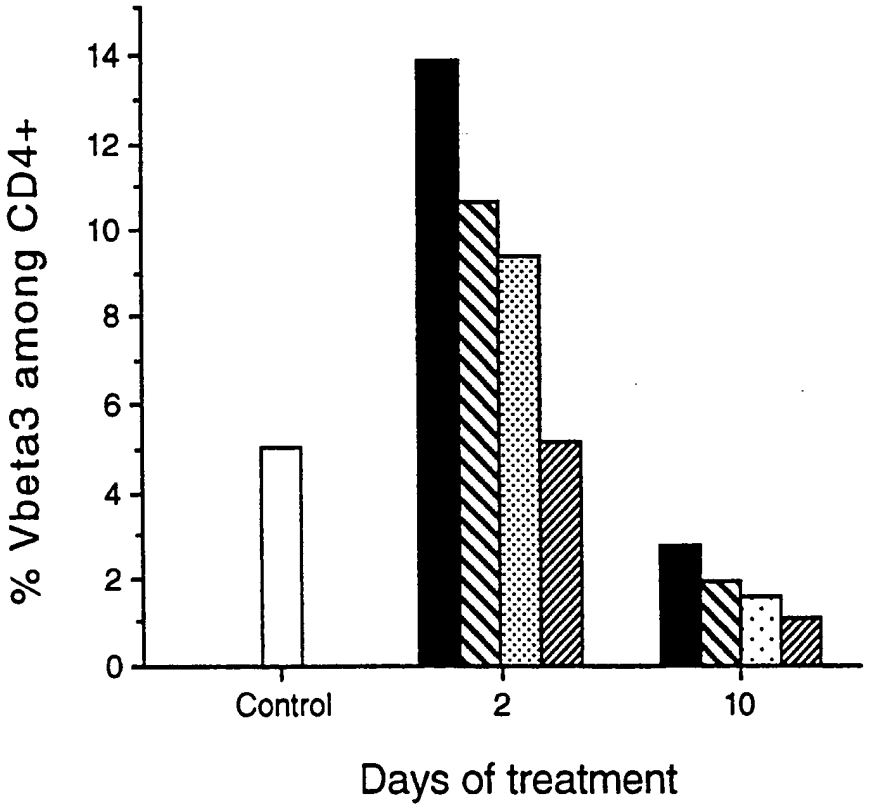
% Vbeta3 among CD4+
0
2
4
6
8
10
12
14
Control
2
10
Days of treatment

FIG. 2A

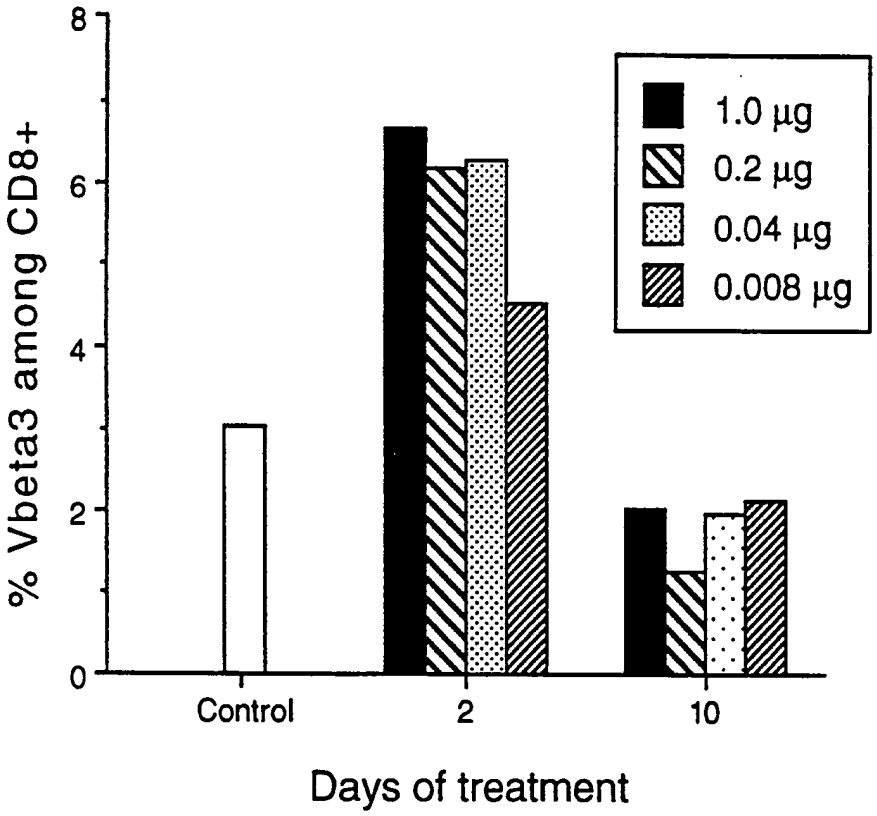
8
6
4
2
0
% Vbeta3 among CD8+
1.0 µg
0.2 µg
0.04 µg
0.008 µg
Control
2
10
Days of treatment

FIG. 2B

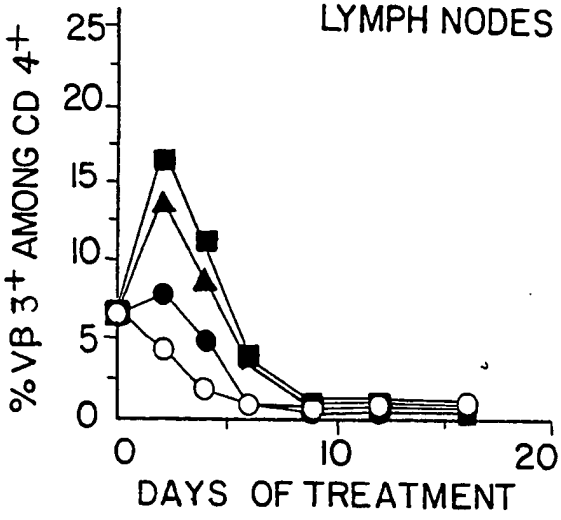


FIG.3A

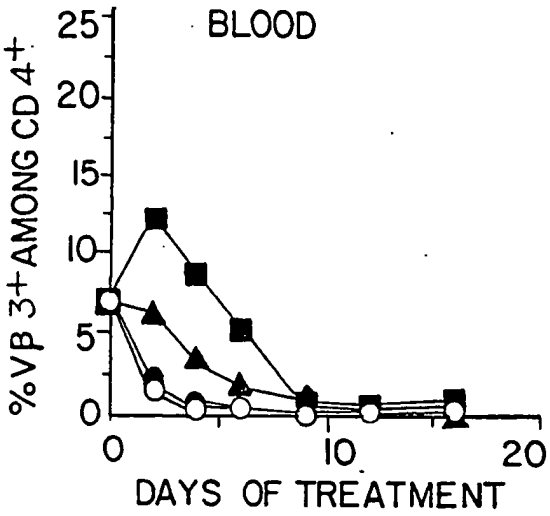


FIG.3B

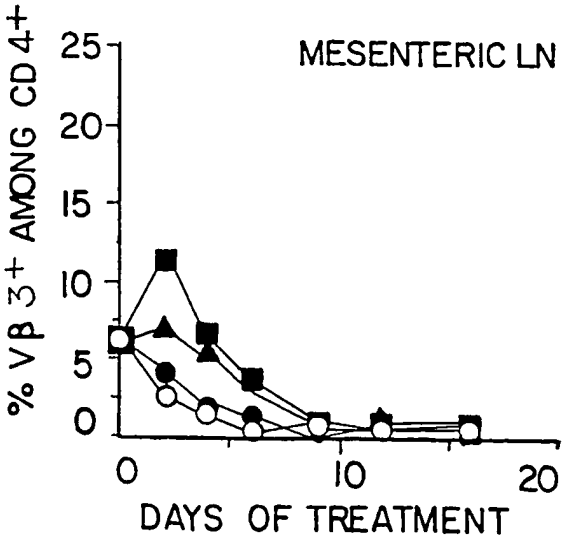


FIG.3C

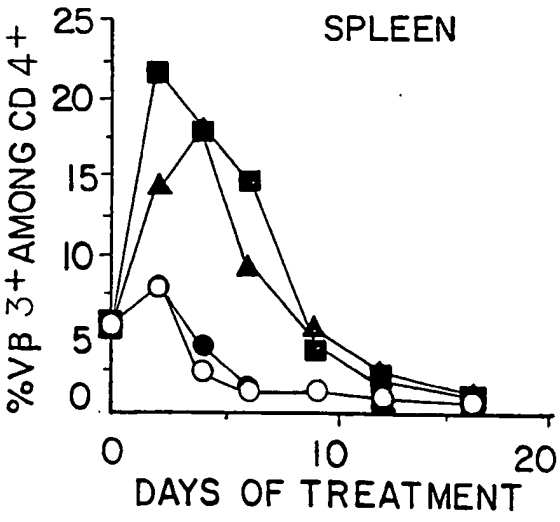


FIG.3D

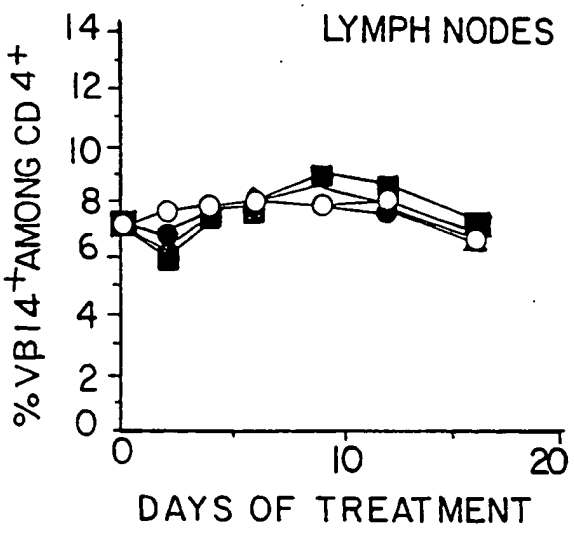


FIG.3E

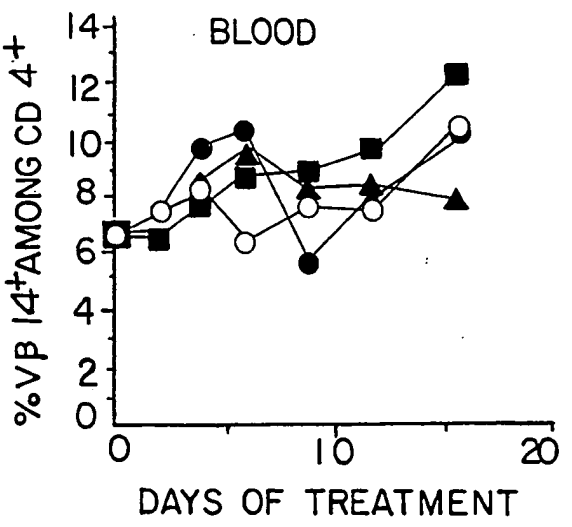


FIG.3F

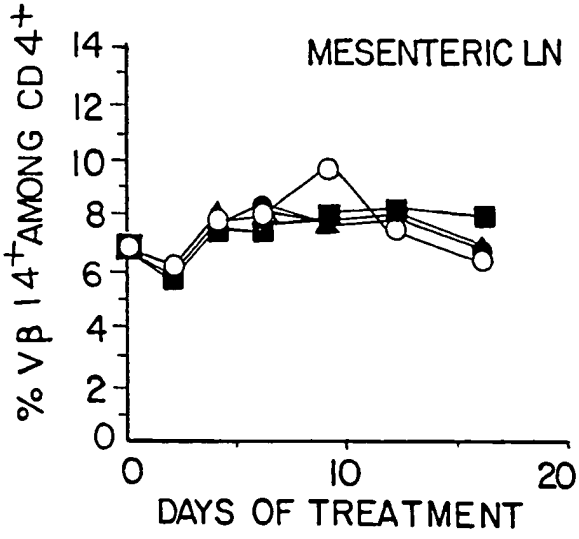


FIG.3G

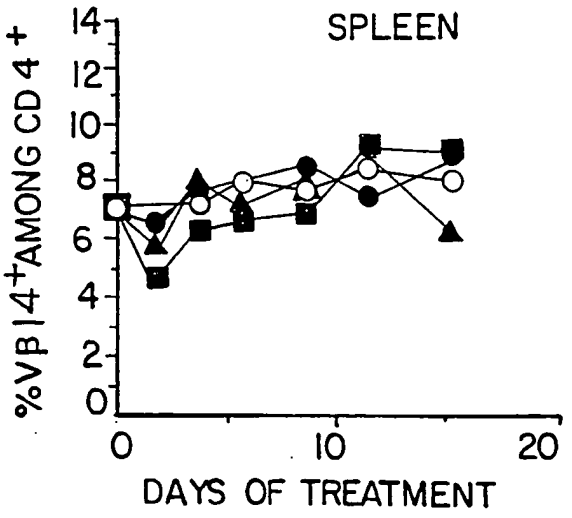


FIG. 3 H

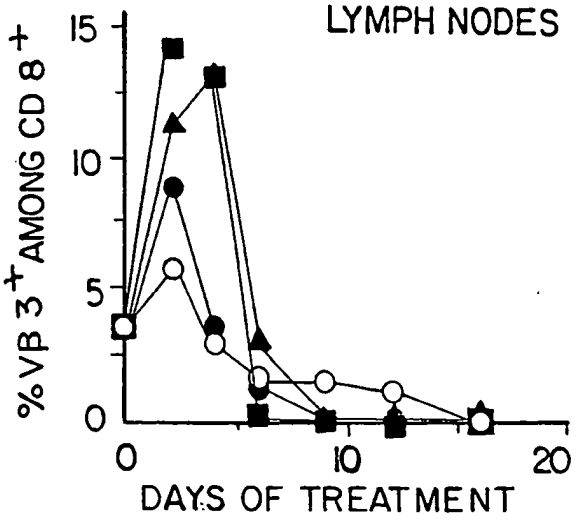


FIG.3I

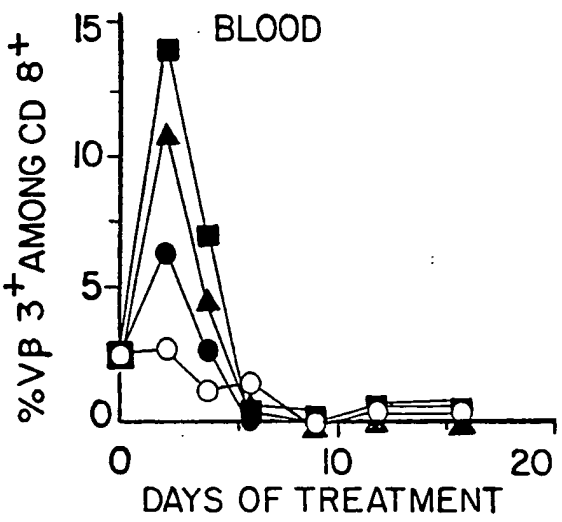


FIG.3J

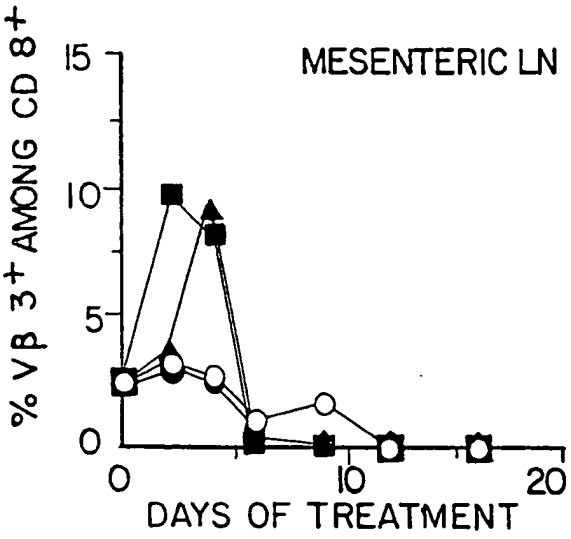


FIG.3K

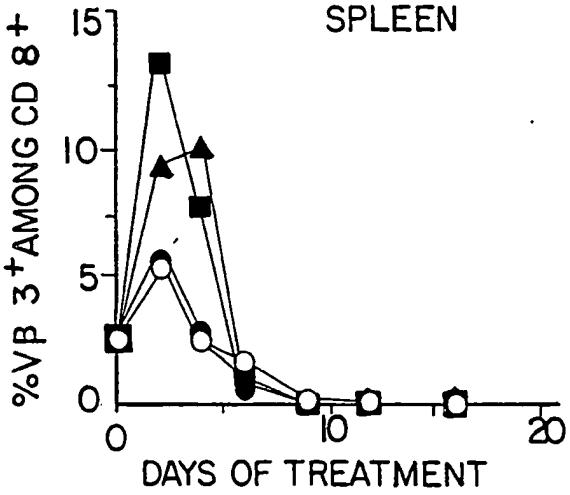


FIG.3L

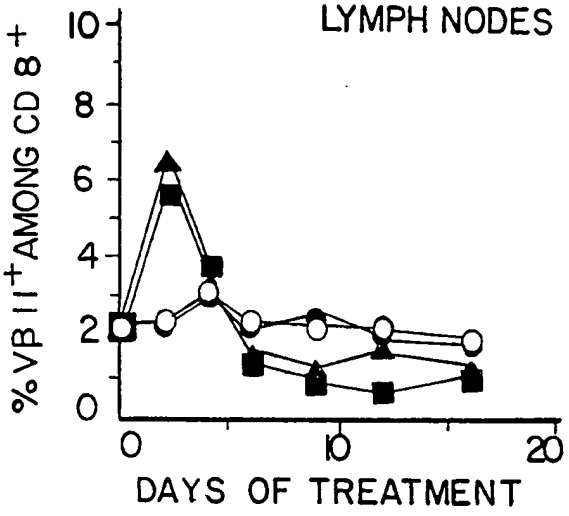


FIG. 3M

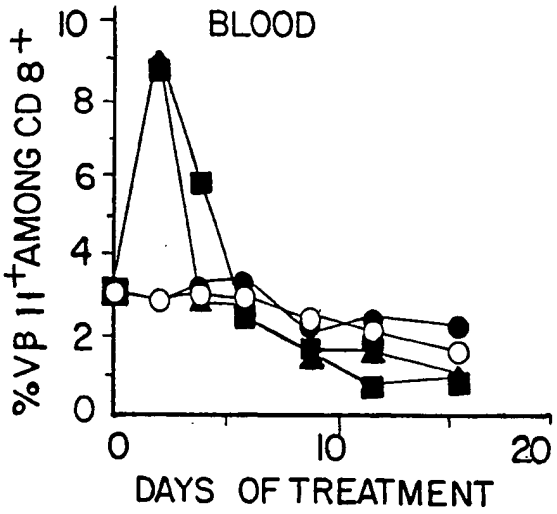


FIG. 3N

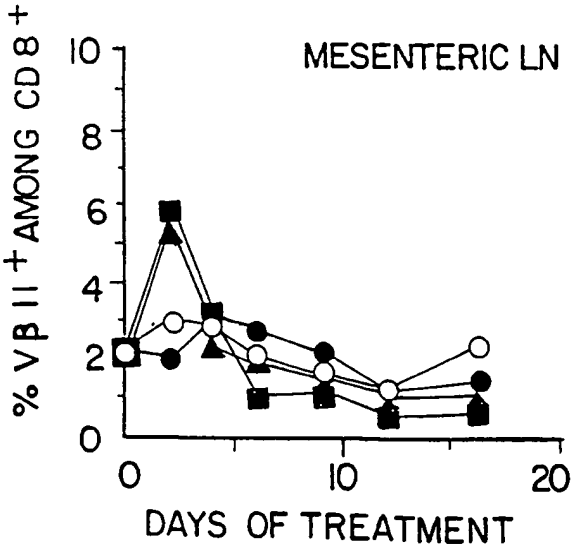


FIG. 3O

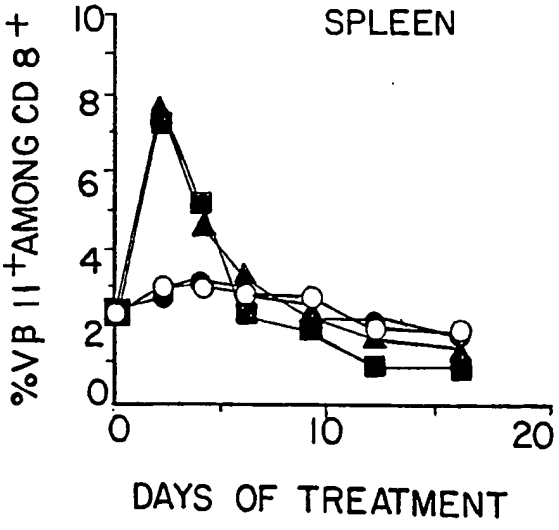


FIG. 3P

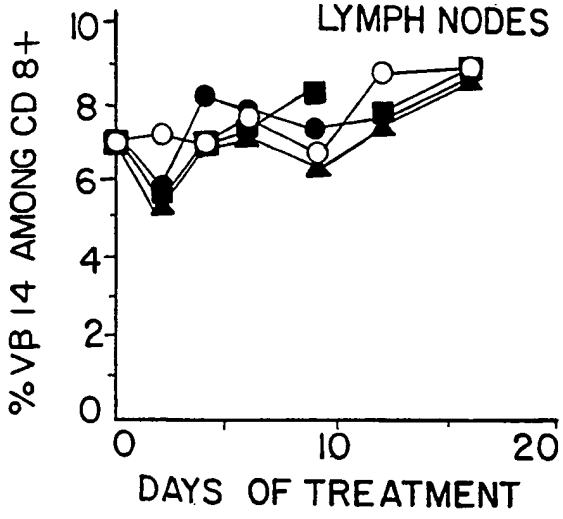


FIG.3Q

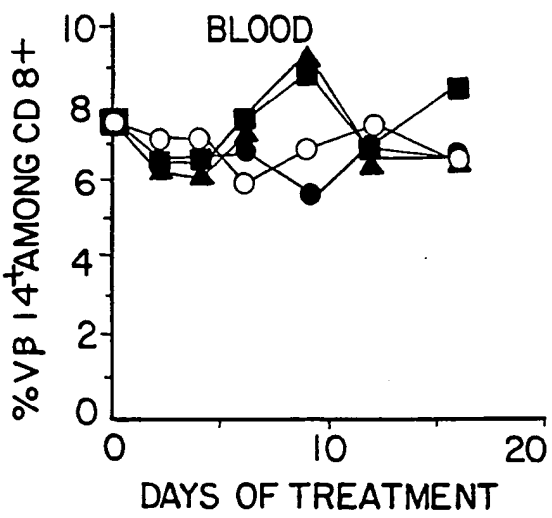


FIG. 3R

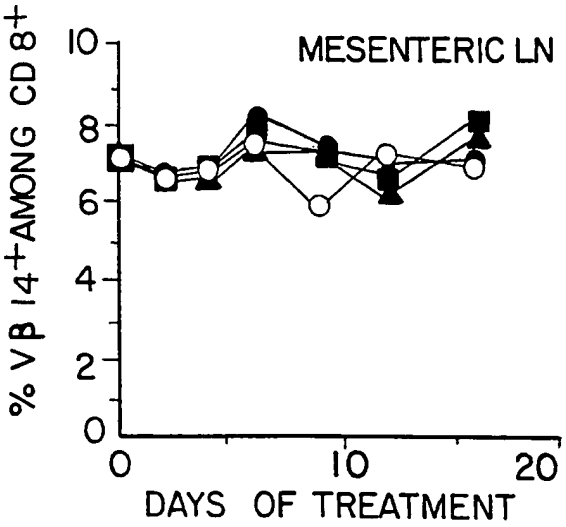


FIG. 3S

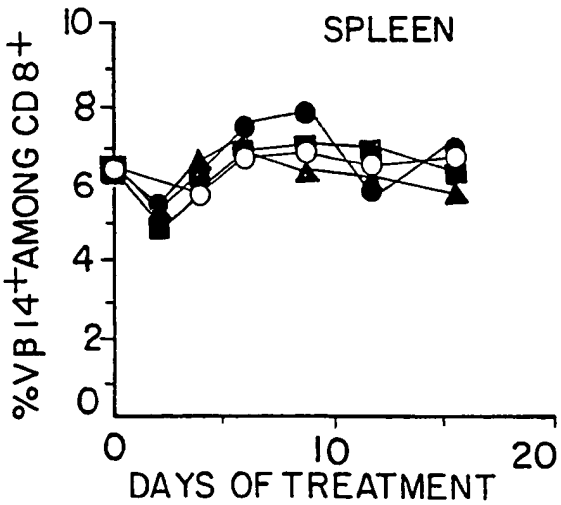


FIG.3T

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02, 39/00, 39/085, 39/12

US CL : 424/88, 89, 92; 514/2; 530/868

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89, 92; 514/2; 530/868

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

530/350, 395

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

AUTOMATED PATENT SYSTEM (APS); DIALOG DATABASE--FILES 155,399, 5, 351

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | Cell, vol. 49, issued 24 April 1987, J. W. Kappler, et al., "A T cell receptor V-beta segment that imparts reactivity to a class II major histocompatibility complex product", see pages 263-271, especially the abstract. | 1-7 |
| Y | J. Exp. Med., vol. 172, issued October 1990, B. L. Rellahan, et al., "In vivo induction of anergy in peripheral V-beta8+ T cells by Staphylococcal Enterotoxin B", see pages 1091-1100, especially the summary. | 1-7 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 MAY 1994

Date of mailing of the international search report

14 JUN 1994

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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | Cell, vol. 54, issued 12 August 1986, J. L. Urban, et al., "Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy", see pages 577-592, especially the abstract. | 1-7 |
| Y | Cell, vol. 54, issued 15 July 1988, H. Acha-Orbea, et al., "Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention", see pages 263-273, especially the abstract. | 1-7 |
| Y | Cell, vol. 56, issued 13 January 1989, J. White, et al., "The V-beta-specific superantigen Staphylococcal Enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice", see pages 27-35, especially the abstract. | 1-7 |